Research Paper | Biochemistry | E-ISSN No : 2454-9916 | Volume : 5 | Issue : 1 | Jan 2019

EFFECTS OF DIABETIC NEPHROPATHY ON PHOSPHOROUS HOMEOSTASIS

Dr. Anjli Pawar [1] | *Dr. Suvarna Prasad [2] | Dr. Rajiv Kumar [1] | Dr. Pallav Sharma [1] | Dr. Sunita Manhas [3] | Dr. Karanpreet Bhutani [3] | Dr Munish Kumar [4]

[1] MD Student, Department of Biochemistry, MMIMSR, Mullana, 133207.

[*2] Professor and Head, Department of Biochemistry, MMIMSR, Mullana, 133207. (*Corresponding Author)

[3] Assistant Professor, Department of Biochemistry, MMIMSR, Mullana, 133207.

[4] MD Student, Department of Forensic Medicine, MMIMSR, Mullana, 133207.

ABSTRACT

A paradoxical metabolic imbalance in inorganic phosphate occurs from the early onset of diabetes and may lead to a reduction of high energy phosphates and tissue hypoxia. These changes take place in the cells and tissues in which the entry of glucose is not controlled by insulin, and particularly in poorly regulated diabetes patients in whom long term vascular complications are more likely to occur. Diabetic nephropathy is one of the most serious complications of diabetes and can lead to glomerulosclerosis and end stage renal diseases. Ultimately resulting in mortality in diabetic patients. This study is an attempt to find out the serum phosphorus levels in diabetic nephropathy patients and to find its association with HbA1c.

KEYWORDS: Diabetes Mellitus, Serum Phosphorus, Diabetic Nephropathy, Microalbuminuria.

INTRODUCTION

Diabetes Mellitus is the most common metabolic disorders with high rate of morbidity characterised by impaired metabolism of glucose and other energy yielding fuels as well as the late development of vascular and neuropathic complications. Diabetes involves group of disorders having different pathogenic mechanism in which hyperglycaemia is the common denominator. Hyperglycaemia has crucial role in the development of diabetic related complications. Some of these are atherosclerosis neuropathy, nephropathy, retinopathy and diabetic foot.[1] There are four potential biochemical pathways linking hyperglycemia to the changes within the kidneys, which can plausibly be linked to the functional, structural changes characterizing the diabetic nephropathy. These are polyol pathway, non- enzymatic glycation, glucose auto oxidation and de novo synthesis of diacylglycerol leading to protein kinase C and phospholipase A2 activation.[2]

Microalbuminuria (MA) was first described in 1982 in patients having diabetes. In this study it was found that there was increased risk of cardiovascular morbidity and mortality in these patients. At the same time, it is accepted as an indicator for the presence of diabetic retinopathy/neuropathy, cardiovascular and peripheral vascular disease and increased mortality. The presence of MA and overt proteinuria in non-insulin dependent diabetes mellitus (NIDDM) is an indicator of poor glycemic control. Increase in prevalence of MA is strongly associated in patients having poor glycemic control, insulin resistance and low Phosphorus levels.[3]

Direct association of macroelements with Diabetes Mellitus have been observed in many different studies. Insulin action has been potentiated by some trace elements such as chromium, magnesium, zinc, manganese and phosphate. Phosphorus is the widely distributed element in the human body.[1] It is present in both organic and inorganic form in the serum but the measurable form is inorganic ion. It has important role for bone mineralisation and cellular structural component like phospholipids, nucleotides, and phosphoprotein. It has been also used as an energy store, for oxygen transport as 2,3 DPG and for its acid base balance. It has been also used in formation of creatine phosphate which is involved in many energy intensive physiological functions, such as muscles contractility, neurological functions and electrolyte transport.[4]

Phosphate is absorbed in the small intestine by both paracellular and active transport. Several factors affect tubular phosphate reabsorption through the sodium phosphate cotransporters located in the tubular cell membrane. Reduced phosphate reabsorption can be due to high intake of dietary phosphate, acidosis, parathyroid hormone, PTH related peptide, glucocorticoid therapy, calcitonin and vitamin D.[5]kidney is the main regulator of phosphorus metabolism.[6]

The present study is designed to find any association and correlation of serum phosphorus level in patients having diabetic nephropathy.

MATERIAL AND METHODS:

This is a cross sectional study approach on diabetic patients. It was conducted in the Department of Biochemistry, MMIMSR, Mullana, Ambala. Patients were enrolled on the basis of following Inclusion and Exclusion criteria.

Inclusion Criteria:

All type 2 diabetic patients, both genders aged 30-55 years.

Exclusion Criteria:

Past medical history of hypertension, chronic renal failure, on renal replacement therapy, regular hemodialysis, malabsorption, heart failure, bone tumors, haematological disorders, chronic diarrhoea and on oral supplements of phosphate.

Study area and study population:

150 diabetic patients aged 30 to 55 years which were further equally subdivided into normoalbuminuria, microalbuminuria and macroalbuminuria and other 50 healthy subjects were included in the study. All subjects signed informed consent and filled questionnaire. The study was approved by the ethical committee of the University.

METHODOLOGY:

Blood samples were collected after 12 hrs fasting period under aseptic conditions. Samples obtained were centrifuged and serum was separated. Serum samples were separated from whole blood collected into the tubes without anticoagulant, after clotting was complete, the tubes were then centrifuged at 2700g for 10 minutes. Serum was removed and assayed for phosphorus by using UV molybedate method on fully automatic EM 360 ERBA Analyser.

HbA1c was measured using EM 360 ERBA analyser. This uses the method using the affinity chromatography. For all the test listed above calibrator used were supplied by kit manufacturer and accuracy of the results obtained for all analytes were validated by using Erba Mannheim accuracy control at 2 different levels. Random urine was taken for estimation of microalbuminuria by Pyrogallol red method, and these values were compared with those of normal healthy subjects.

Statistically Analysis:

For statistical analysis of data, software downloaded from the website was used to calculate correlation coefficient (r), student's distribution (t) and probability (P) between HbA1c and Phosphorus.

RESULTS AND OBSERVATIONS:

The mean age of cases and controls were 46.65(78 males and 72 females) and 44.63 years (with 28 males and 22 females),) respectively which was statistically insignificant (Figure I). The maximum number of patients was in the age group of 46-50 i.e. 32% (Figure II) for both cases and controls. 84% of control showed a normal serum phosphorus level in 2.5- 4.5 mg/dl. And in diabetic cases 40.67% had decreased level than the normal range i.e. < 2.5 mg/dl and 54.67% of them in normal range and remaining 4.67% were above than normal levels i.e. > 4.5 mg/dl (Figure III). As shown in Figure IV & Table I, Serum Phosphorus levels of Group IB and IC has significant association with Group II. Also significant association between Group IB and IC was found. As seen in Table II that correlation between Serum HbA1c and Serum Phosphorus of Group IA was moderately negative and statistically significant. However correlation for Group IB and Group IC was negative but it is weakly correlated statistically.

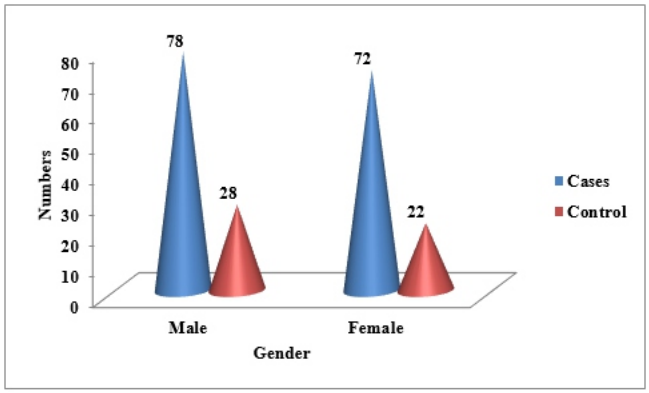

Figure I. Gender wise distribution of Sub-groups in Cases and Control

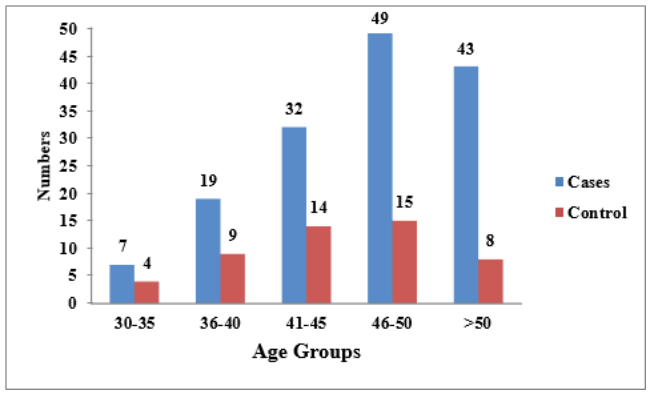

Figure II. Age wise distribution of Sub-groups in Cases and Control

P<0.001

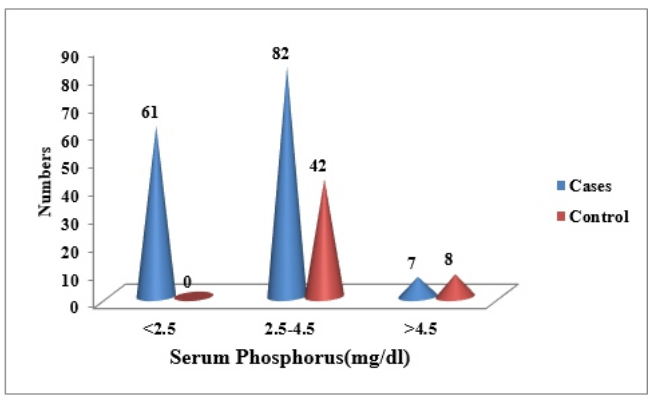

Figure III. Serum Phosphorus level in Cases and Control

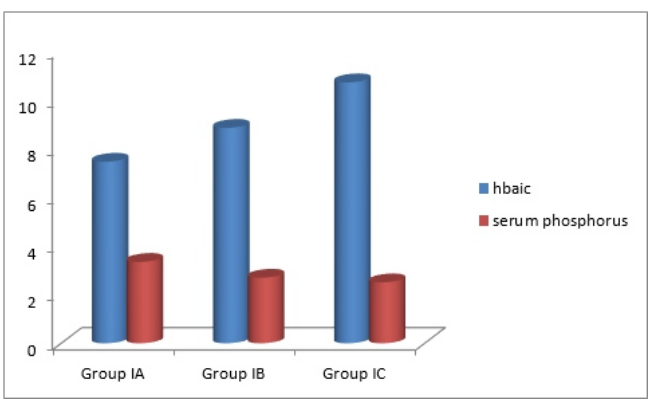

Figure IV: Comparison of Serum phosphorus between Group IA, IB, IC and II

Table I: Comparison of Serum phosphorus between Group IA, IB, IC and II

Parameters	Group	Mean	Standard Deviation	P value
Serum Phosphorus	Group IA	3.24	0.52	0.00032
	Group II	3.82	0.61	
	Group IB	2.72	0.41	<0.001*
	Group II	3.82	0.61	
	Group IC	2.38	0.31	<0.001*
	Group II	3.82	0.61	
	Group IA	3.24	0.52	0.004
	Group IB	2.72	0.41	
	Group IA	3.24	0.52	0.04
	Group IC	2.38	0.31	
	Group IB	2.72	0.41	<0.001*
	Group IC	2.38	0.31	

Table II. Correlation between Serum HbA1c and Serum Phosphorus within Groups IA, IB and IC

Group	Parameters	Mean	Standard Deviation	Karl Pearson's Correlation Coefficient (r)
Group IA	Serum HbA1c	7.57	0.67	-0.46
	Serum Phosphorus	3.24	0.52	
Group IB	Serum HbA1c	8.53	0.69	-0.12
	Serum Phosphorus	2.72	0.41	
Group IC	Serum HbA1c	10.07	1.18	-0.02
	Serum Phosphorus	2.38	0.31	

DISCUSSION:

Out of the 200 subjects studied, 150 which were diabetic had 78 (52%) males and 72 (48%) females. Raul et al reported 52.73% males and 47.27% females in their study.[4] Willer et al and Rao et al, in their studies also shows male preponderance which is statistically insignificant.[7,8]

In our study, the maximum number of patients was in age group of 46-50 years. Age mean of diabetic patients was 46.45±6.55. In our study, the mean phosphorus levels in diabetic patients were 2.84±1.1 which was lower as compared to controls (3.84±0.66). A significant association between the group IA, IB with Group II, Group IB with Group IC was found for the serum phosphorous(Table I). Many studies have also found decreased concentration of phosphate in poorly regulated diabetic patients and the level increases when blood glucose is controlled.[9]

According to Gartner et al study in juvenile onset of diabetic patients found that as plasma glucose decreased from 221 mg/dl to 95.5 mg/dl, serum inorganic phosphorus was rose from 4.09 to 5 mg/dl.[10] In our study, there is decreased phosphorus concentration in the diabetics and as the disease progresses level of phosphorus further deteriorate. This is in concordance with a study done by Revathi [1] and Ugwuja.[11] Findings of the earlier studies are in concordance with our work which can be due to osmotic diuresis and intracellular shift.[9]

As osmotic diuresis is the most common factor for the enhanced urinary secretion and decreased electrolytes, while intracellular shift can also be responsible.[12] Osmotic diuresis in hyperglycaemic and acidemic states may cause a competition between phosphate and glucose for excretion in proximal tubular system. Uncontrolled diabetics have glucose accompanied by phosphate into the cells, resulting in low blood phosphorous levels.[13] Dietzel et al found that maximal capacity of renal tubular reabsorption of phosphate was significantly suppressed in diabetic patients and also reported that urinary phosphate excretion was three times higher in diabetic patients when compared to healthy controls.[6,14]

Sultan et al revealed that there is decreased serum calcium which is due to hyperglycemia that enhances calcium and phosphorus excretion in urine.[15] Hyperglycemia causes excess urinary phosphate in patients with type 2 diabetes mellitus. Nagasaka et al suggested that hyperglycemia caused excess urinary cal-

cium and phosphorus excretion in patients with NIDDM.[16] Serum phosphoprus levels were decreased in type 2 diabetic patients indicating that there is alteration in the phosphorus metabolism ,represented by the study done by Zhong et al.[17] Kalaitzidis et al observed that patients with metabolic syndrome showed significantly lower phosphate and magnesium levels compared with controls.[18] Raskin and Pak studied 21 diabetic patients in whom treatment results ranged from "suboptimal" to "optimal" control and found that, as the mean plasma glucose decreased from 17.1 mmol/L to 5.2 mmol/L over 4 to 10 days, serum phosphate level raised from 1.12 to 1.26 mmol/L.[19]

A conflicting metabolic variance in phosphate occurs from early onset of diabetes and may lead to a reduced high energy phosphate and hypoxia of tissues. These variations take place in the cell and tissues in which access of glucose is not regulated by insulin and specifically in poorly controlled diabetic patients in which chronic vascular complications are more likely to occur. Decreased serum phosphate level are related with severity of diabetes mellitus.[14]

A disturbance in phosphate regulation occurs in kidney nephrons, where the increased sodium dependent glucose entry in diabetics impairs phosphate reabsorption. Glucose is more potent than inorganic phosphate in stimulating the uptake of sodium in the renal microvillus vesicles. The elevated glucose concentration depolarize the transmembrane electrochemical sodium gradient of the brush border membrane for inorganic phosphate entry into the tubular cells and decrease intracellular phosphate leading to hyperphosphaturia.[6,20] This can cause decreased level of phosphate in the diabetics and normalization of blood glucose level improve the capacity of kidney tubules to reabsorb phosphate and ultimately increase phosphate concentration. It is also suggested by the DCCT and UKPDS studies that improved glucose regulation definitely influence or also prevent the long term diabetic complications. [21,22] This decreased urinary phosphorus loss can be due to reduced glycosuria and affected by direct action of insulin on renal tubules or suppression of glucagon and parathyroid hormones secretion.[13]

Haglin et al[23] in 2001, Park W et al[24] in 2009 showed the negative correlation between serum phosphate levels and fasting blood sugar levels. In our study, a significant negative correlation of phosphorus and HbA1c was found in T2DM patients.

CONCLUSION:

The inorganic phosphate is essential for the resynthesis of 2,3 diphosphoglycerate and ATP, therefore phosphate depletion results in tissue hypoxia and decrease in energy rich phosphate with disturbances of various organ systems. Our findings suggest that there is a low phosphorus level in type 2 diabetes mellitus. Increased microalbuminuria was seen with reduced glucose tolerance hence early estimation of both the parameters should be done while monitoring the cases of type 2 diabetes and thus will help to decrease the incidence of renal failure. Phosphorus depletion may increase the risk of secondary complications, preventing low phosphorus in diabetics can be beneficial in the management of the disease. Regular control of plasma phosphate levels and prophylactic substitution of phosphate are recommended.[25]

REFERENCES

1. Revathi R, Amaldas J.(2014) A Clinical study of serum phosphate and magnesium in type II diabetes mellitus. Int J Med Res Health Sci, 3(4): 808-12.
2. S.R Salaqh Hamed, P Pavkovic.(2000) Microalbuminuria and diabetes mellitus. Diabetologia Croatia.31(4):209-21.
3. Arpaci D, Tocoglu AG, Ergenc H, Korkmaz S, Vcar A, Tamer A. (2015) Association of Serum Magnesium level with DM & Diabetic complications. Hippokratia. 19(2): 153-7.
4. Raul KM , Tungikar S, Soni N, Deshpande S, Raundal S. (2015) Study of serum phosphorus levels and its correlation with clinical profile in patients with diabetic ketoacidosis. IOSR- JDMS. 14(5):1-10.
5. Laroche M, Boyer JF. (2005) Phosphate diabetes, tubular phosphate reabsorption & phosphotonins. Joint, bone, spine revere du rhumatisme.72(5):376-81.
6. Ditzel J, Lervang HH. (2009) Disturbance of inorganic phosphate metabolism in diabetes mellitus: temporary therapeutic intervention trials. Diabetes Metab Syndr Obes. 2: 173–7.
7. Willer A, Harreiter J, Pacini G. (2016) Sex and Gender differences in risk, pathophysiology and complications of Type 2 Diabetes Mellitus. Endocr Rev. 37(3): 278-316.
8. Rao DV, Pradhan B, Mallikarjuna Y, Reddy R. (2012) Clinical profile of diabetic ketoacidosis in adults. Nepal Journal. 10(2): 80-6.
9. Bora G, Rajakakati R, Kakati S, Yadav S. (2016) serum inorganic phosphate concentration and glycated hemoglobin percent in type 2 diabetes mellitus. IJHSR. 6(10):96-104.
10. Gertner JM, Tamborlane WV, Horst RL.(1980) Mineral metabolism in diabetes mellitus: changes accompanying treatment with a portable subcutaneous insulin infusion system. Journal of clinical endocrinology and metabolism.5(5): 862-6.
11. Ugwuja E, Eze N.(2006). Comparative study of serum electrolytes, total protein, calcium and phosphate among diabetic and HIV/AIDS patients in Abakaliki, South eastern Nigeria. The Internet Journal of Laboratory Medicine. 3(2): 1.
12. Hebden RA, Gardner SM,Benett T,Mac Donald IA.(986). The influence of stertozocin-induced diabetes mellitus on fluid and electrolytes in handling in rats. Clin.Sci. 70(1):111-7.
13. Haap M, Hellar E, Thamer E, Tschritter O, Stefan N, Fritsche A.(2006). Association of serum phosphate levels with glucose tolerance, insulin sensitivity and insulin secretion in non-diabetic subjects. European journal of clinical nutrition .60:734-9.
14. Najeeb Q, Aziz R, Hamid S.(2014). To evaluate the levels of glycated hemoglobin, serum calcium, magnesium, phosphate, uric acid and microalbuminuria in patients with newly diagnosed type 2 diabetes mellitus. Int.J Res Med Sci. 2(4):1462-5.
15. Sultan, E.; Taha, I.; Saber, LM. (2008) Altered Bone Metabolic Markers In Type 2 Diabetes Mellitus: Impact of Glycemic Control. Journal of Taibah University Medical Sciences. 2: 104-16.
16. Nagasaka S, Murakami T, Uchikawa T, Ishikawa SE, Saito T. (1995) Effect of glycemic control on calcium and phosphorus handling and parathyroid hormone level in patients with non-insulin-dependent diabetes mellitus. Endocrine Journal. 42(3):377-83.
17. Zhong N, Xue Xue D, Bao Y, Xue B.(2016) Level of phosphorus and adult type 2 diabetes mellitus. J Cent South Uni (Med sci.). 41(5):502-6.
18. Kalaitzidis R, Tsimihodimos V, Bairaktari E, Siamospoulos KC, Elisaf M. (2005) Disturbances of phosphate metabolism: another feature of Metabolic Syndrome. American journal of kidney diseases. 45(5):851-8.
19. Raskin P, Pak CYC. (1981) The effect of chronic insulin therapy on phosphate metabolism in Diabetes Mellitus. Diabetologia. 21(1): 50-3.
20. Barrett PQ, Aronson PS. (1982) Glucose and alanine inhibition of phosphate transport in renal microvillus membrane vesicles. Am J Physiol. 242(2): F126-F131.
21. The diabetes control and complications trail research group. The effect of intensive treatment of diabetes in insulin dependent diabetes mellitus. N Engl J Med. 1993; 329(14): 977-86.
22. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood glucose control with sulfonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. (UKPDS 33). Lancet 998; 352(9131):837-53.
23. Haglin L, Lindblad A, Bygren LO.(2001) Hypophosphataemia in the metabolic syndrome. Gender differences in body weight and blood glucose. Eur J Clin Nutr. 55(6): 493-8.
24. Park W, Kim BS, Lee JE, Huh JK, Kim BJ, Sung KC, et al.(2009) Serum phosphate levels and the risk of cardiovascular disease and metabolic syndrome; a double edged sword. Diabetes Res Clin Pract.83(1): 119-25.
25. Arun kumar D, Revathi K, Rajeshwari S, Swaminathan S. (2015). The diagnostic significance of calcium, phosphorus, magnesium and uric acid in type 2 diabetes mellitus and their association to HBA1C. J Chem Pharm Res. 7(8):390-7.